Instrucciones rápidas del Fluorímetro

- Encima del equipo no debe de haber ningún tipo de reactivo (en general, NADA).
- La tapa ha de estar SIEMPRE cerrada, sólo debe abrirse para cambiar las cubetas.
- En el cajón que está bajo llave se guardan las cubetas.
- Las cubetas se han de limpiar después de cada uso con agua destilada y después con Etanol.
- Registrar las horas que está encendida la lámpara. SIEMPRE!!

ENCENDIDO DEL EQUIPO:

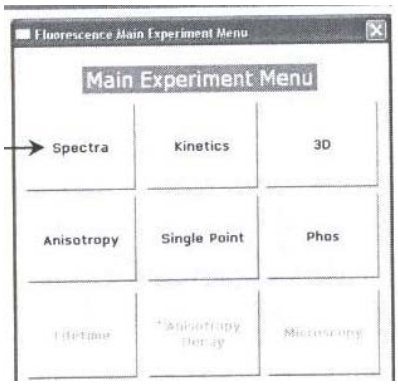
MUY IMPORTANTE HACERLO SIEMPRE EN ESTE ORDEN

1. Conectar el cable de alimentación de detrás de la torre Spectra CQ (en el suelo)
2. Encender el ordenador Spectra CQ
3. Encender el interruptor del ventilador (por detrás del equipo, interruptor inferior)
4. Encender la lámpara del equipo (por detrás del equipo, interruptor superior)
5. Esperar a que el equipo haga dos pitidos (unos 2 min)
6. Encender el ordenador de sobremesa
7. Ejecutar el programa de FluorEssence (Origin Lab).
8. Hacer Click en la tecla "Menu" del programa (M). En este momento, se realizará una comprobación del sistema.

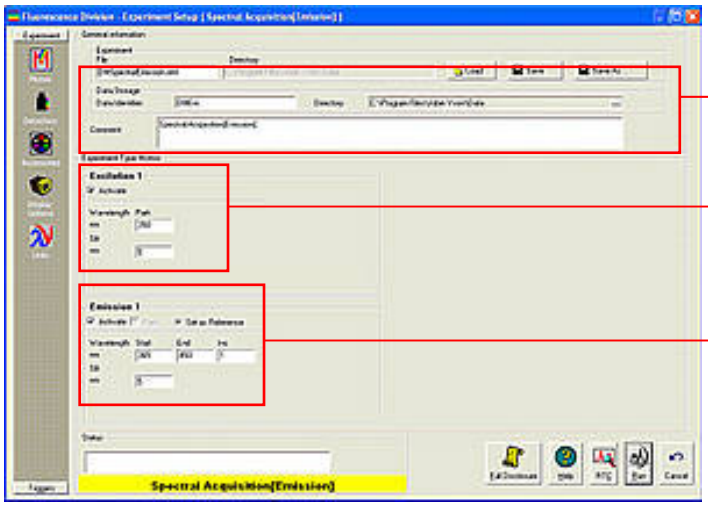
IMPORTANTE: entre cada paso esperar unos segundos. Sino, al inicial el programa la comprobación del equipo dará error y se tendrá que reiniciar todo el sistema.

PARA REGISTRAR ESPECTROS DE EMISIÓN:

1. Al hacer click en la tecla "Menu" del programa (M), nos aparecerá un cuadro con varias opciones. Seleccionar "Spectra".



2. Aparecerán 3 opciones: Emission , Excitation y Synchronous. Seleccionar la opción de Emission.
3. En la ventana Experiment Setup seleccionaremos los parámetros.



Información sobre la muestra.

Longitud de onda de excitación

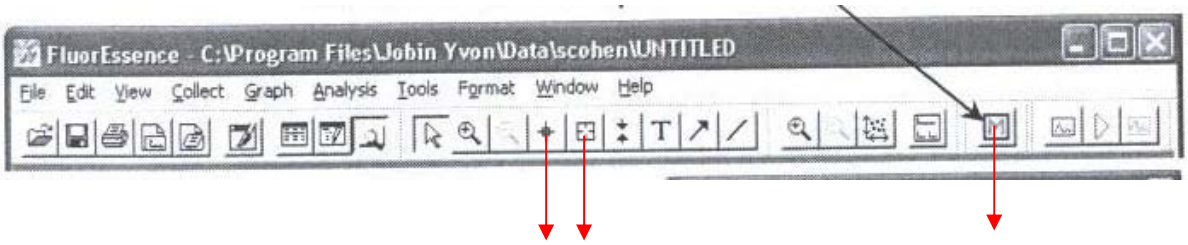
Rango de longitudes de onda del espectro. Por ejemplo, si excitamos a 500 nm, el rango de longitudes de onda podría ser de 510 a 800 nm. Es aconsejable dejar un margen de unos 10 nm respecto la λ exc y el inicio del rango de emisión. Normalmente, el valor del Slit se fija a 5.

4. Una vez seleccionados los parámetros, hacer click en la tecla "Run".
5. Después de registrar el primer espectro, el programa pedirá el nombre del proyecto.

PARA REGISTRAR ESPECTROS DE EXCITACIÓN:

1. Al hacer click en la tecla “Menu” del programa (M), nos aparecerá un cuadro con varias opciones. Seleccionar “Spectra”.
2. Aparecerán 3 opciones: Emission , Excitation y Synchronous. Seleccionar la opción de Excitation.
3. En la ventana Experiment Setup seleccionaremos los parámetros. Seleccionar los parámetros de manera equivalente a los de emisión. Ejemplo: Emission wavelenth 550 nm (si el máximo de emisión fuera 550nm). Rango del espectro de excitación: 350-540 nm. Slits a 5 nm.
4. En este tipo de espectros es necesario seleccionar otro tipo de detector. En la pestaña de la izquierda, en “Detectors”, añadir S1/R1 y eliminar el resto.
5. Comprobar que el resto de variables en la pestaña de “Monos” no han variado. Hacer click en Run.

ICONOS BÁSICOS ÚTILES:



MENU PRINCIPAL

Haciendo click es estos botones se puede seleccionar cualquier punto del espectro. Los valores de intensidad y longitud de onda de ese punto punto se pueden visualizar en el recuadro de resultados.

INFORMACIÓN ÚTIL SOBRE EL PROGRAMA:

El programa guarda los ficheros en proyectos. Un proyecto contiene hojas de datos, gráficas y notas.

Los gráficos pueden abrirse directamente con el programa Origin Lab sin necesidad de importar los datos. Dentro del proyecto, los worksheets se grabarán en la carpeta “Data”.

APAGADO DEL EQUIPO:

MUY IMPORTANTE HACERLO SIEMPRE EN ESTE ORDEN

En general, el apagado del equipo es exactamente igual que el encendido pero a la inversa.

1. Cerrar el programa FluorEssence
2. Apagar el ordenador de sobremesa
3. Apagar la lámpara del equipo (por detrás del equipo, interruptor superior)
4. NO APAGAR EL VENTILADOR
5. Desconectar el ordenador Spectra CQ
6. Desconectar el cable de alimentación de detrás de la torre Spectra CQ (en el suelo)
7. PASADOS AL MENOS 15 MIN, DESCONECTAR EL VENTILADOR (por detrás del equipo, interruptor inferior)
8. Registrar el tiempo de encendido de la lámpara.

Más abajo, se pueden ver imágenes escaneadas del Manual de Instrucciones Rápido.

MANTENIMIENTO:

Empresa: HORIBA JOBIN YVON
Persona de contacto: Alberto Lozano [alberto@mtb.es]

Mantenimientos realizados:

Fecha	Trabajo realizado	Técnico	Importe total

XENON LAMP RECORD OF USE

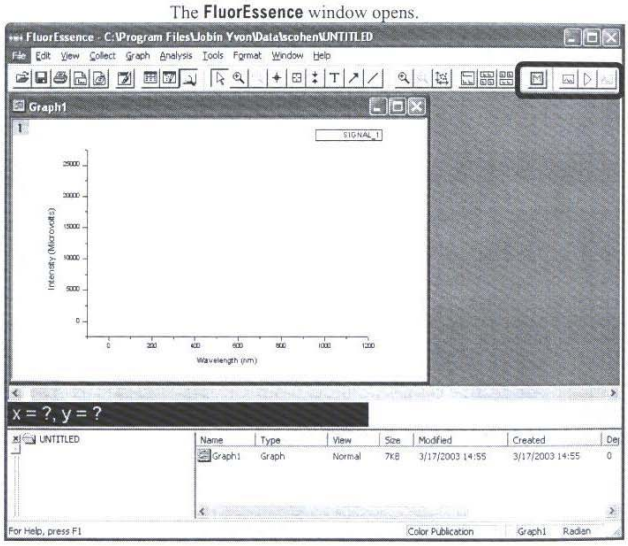
Operator	Date	Time On	Time Off	Incidence	Total Time
TOTAL HOURS					

2: Quick Guide to Running a Scan

1 Turn on the host computer, and all instruments and accessories, as explained in their respective instruction manuals.

2 Click on the FluorEssence short-cut to start FluorEssence™.

The **FluorEssence** window opens.

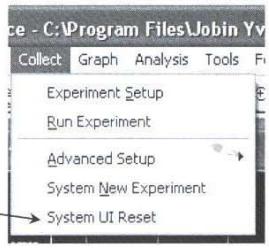


There are four special buttons for running experiments in FluorEssence™:

Experiment Menu		Choose an overall type of experiment to run, such as Phosphorescence, general Spectra (e.g., emission or excitation), or Single Point.
Previous Experiment		Modify slightly a previously set-up experiment, and run it.
Auto Run Previous Experiment		Run a previously set-up experiment without modification.
Make Overlay File		With an existing graph selected, creates an `.SPC` file for use as an overlay file. The existing graph should contain a single spectrum.

From any of these buttons, *upon initial start-up of the software*, you can choose a hardware configuration, and experiment type. After a hardware configuration is loaded, each button has its own separate function.

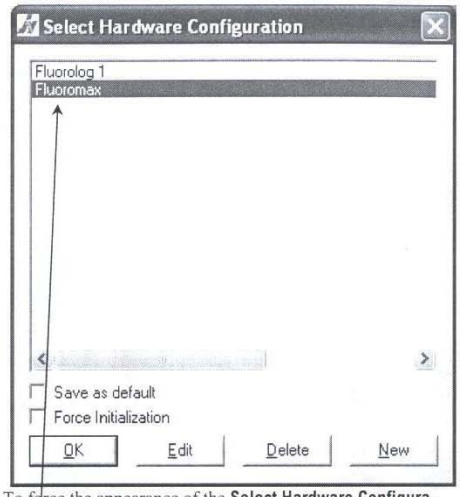
The Collect menu near the top of the main window also has these functions, plus another important command, System UI Reset. In case the four special buttons are grayed out, choose the System UI Reset command. See *Chapter 5: Technical Support* for more details.



3 Click the Experiment Menu button.

The **Select Hardware Configuration** window opens.

Note: *This window does not appear if you have only one hardware configuration installed. Skip to page 25.*

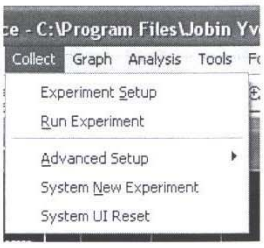


To force the appearance of the **Select Hardware Configuration** window:

a Immediately upon opening FluorEssence™, press F8 and simultaneously click the Experiment Menu button, .

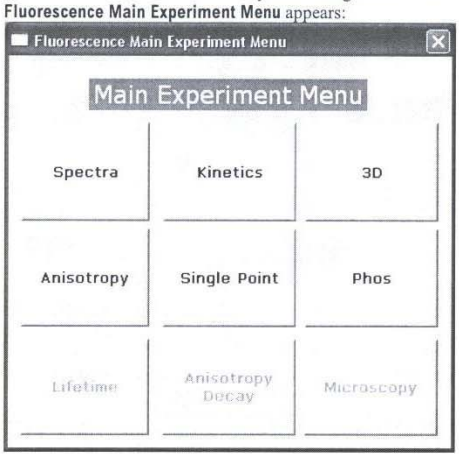
Or

b At any time within FluorEssence, press F8 while choosing the Collect Menu / Advanced Setup / System Reinitialization.



4 Choose a system configuration from the list, then click OK.

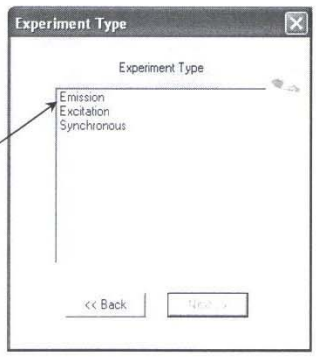
FluorEssence™ loads the chosen system configuration. The **Fluorescence Main Experiment Menu** appears:



5 Click an available scan type, say, Spectra.

The **Experiment Type** window appears.

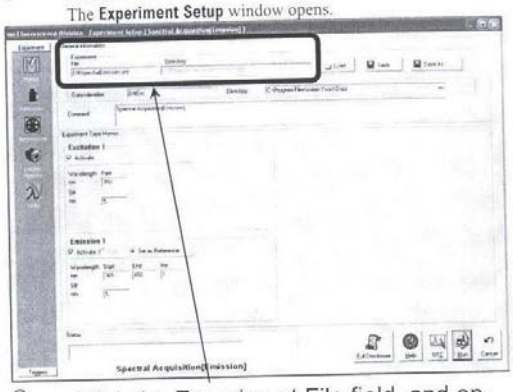
6 Choose an Experiment Type from the menu, say, Emission.



The parameters required for an emission scan are loaded into the **Experiment Setup** window, which appears. For an emission scan, the default parameters are a water-Raman scan.

7 Click Next >>.

The **Experiment Setup** window opens.



8 Click the Experiment File field, and enter a new file name or select a previously saved file.

9 Verify that the experimental parameters are correct.

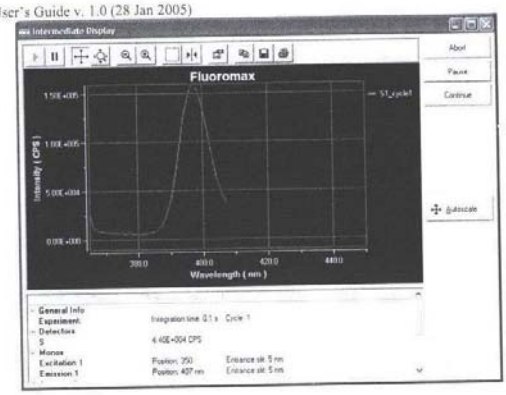
Be certain to check all parameters under all icons in the left-hand column.

10 Insert the sample into the sample compartment, and close the cover of the sample compartment.

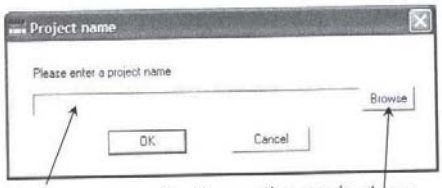
11 Click Run.

The collected spectrum is displayed on the **Intermediate Display** screen:

Note: *If the scan is extremely fast, the* **Intermediate Display** *may be only incompletely or rapidly displayed before the Origin window appears.*

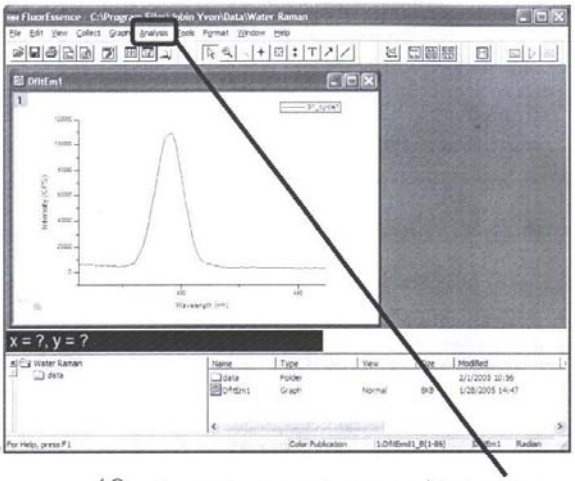


You can watch the incoming data in real time, along with how the positions of accessories vary. The scan may be paused, continued, or aborted. After all data are recorded, the **Intermediate Display** vanishes. For a new project, the **Project name** window appears:



12 Enter a name for the entire project, or browse for an existing project name with the Browse button, then click OK.

All data are moved to Origin®'s graph window:



13 Do post-processing as needed, using the Analysis menu.

3: Tips & Tricks

Calibration of your instrument

Excitation calibration

Monochromator parameters for the xenon-lamp scan:

Monochromator (1200 grooves/mm)	Initial wave-length	Final wave-length	Incre-ment	Slits (band-pass)
Excitation	200 nm	600 nm	1 nm	1 nm
Emission	350 nm	--	--	1 nm

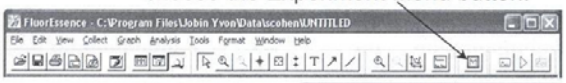
Detector parameters for the xenon-lamp scan:

Detector (Signal)	Integration time	Units
Signal (S1)	100 ms	CPS
Reference (R1)	100 ms	mA

1 Close the sample compartment's lid.

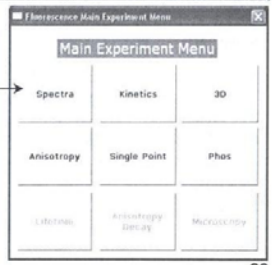
2 Start FluorEssence™.

3 In the main FluorEssence window, choose the Experiment Menu button.



The **Fluorescence Main Experiment Menu** appears.

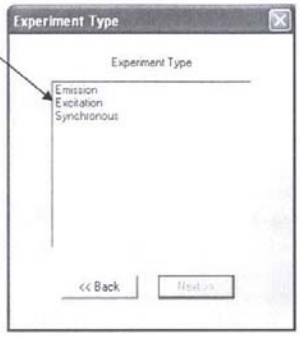
4 Choose Spectra.

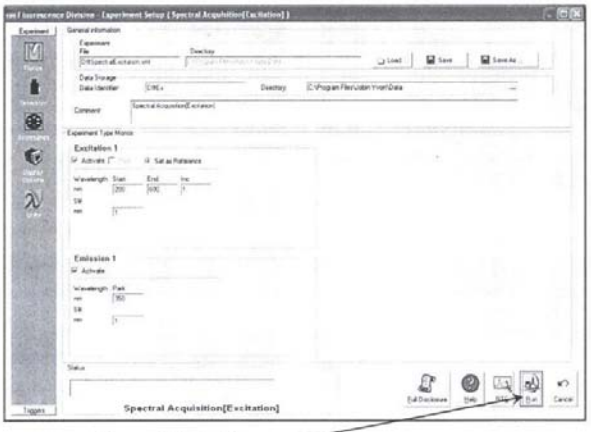


The **Experiment Type** window appears.

5 Choose Excitation.

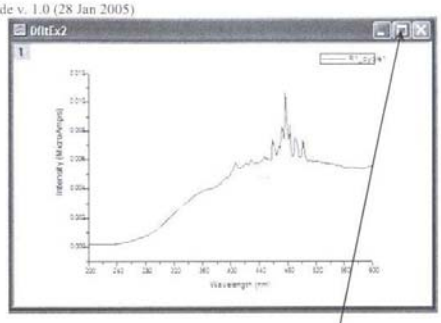
The xenon-lamp scan automatically loads.





6 Click Run.

The **Intermediate Display** opens. The xenon-lamp scan runs:

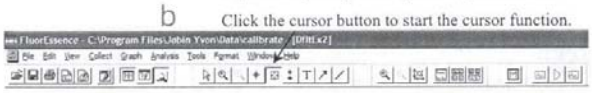


Above is an uncalibrated FluoroMax® lamp-scan. The main peak ought to be at 467 nm, but here appears near 480 nm.

7 Calibrate the excitation monochromator, if required.

a Expand the plot by clicking the Expand button.

b Click the cursor button to start the cursor function.

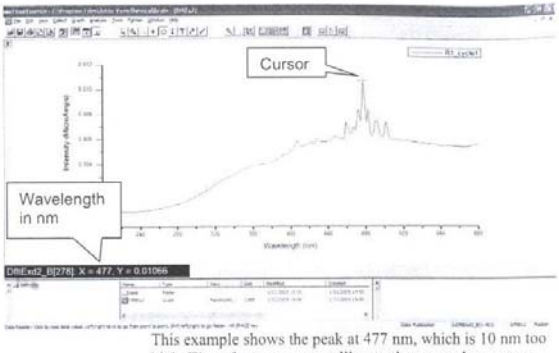


c Click on the graph near the peak, to place the cursor on the graph.

d Using the left and right arrows on the keyboard, move the cursor to the top of the peak.

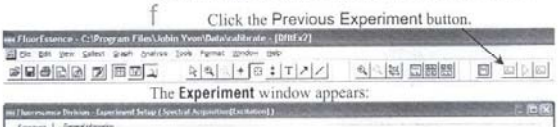
e Read the *x*-value of the plot: this is the wavelength of the peak:

***Note:** Your lamp scan may appear different, depending on the instrument and its configuration.*



This example shows the peak at 477 nm, which is 10 nm too high. **Therefore we must calibrate the monochromator.**

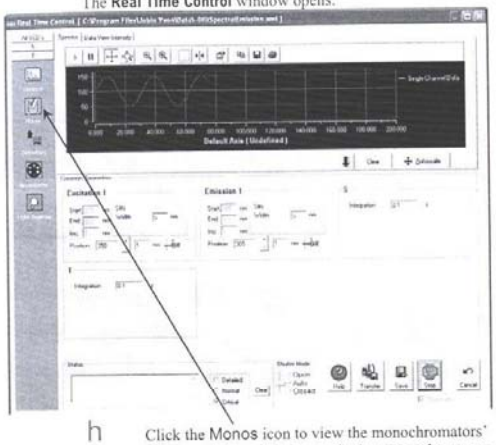
f Click the Previous Experiment button.



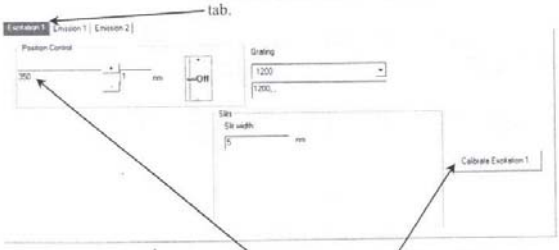
The **Experiment** window appears:

g Click the RTC button on the lower right.

The **Real Time Control** window opens.



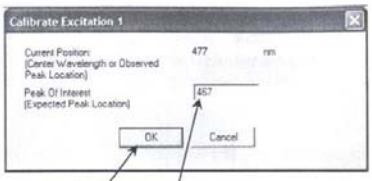
h Click the Monos icon to view the monochromators' index card, then click the excitation monochromator tab.



i Enter the current, observed position of the peak in the Position Control (here, 477 nm).

j Click Calibrate Excitation 1.

The **Calibrate** window opens:

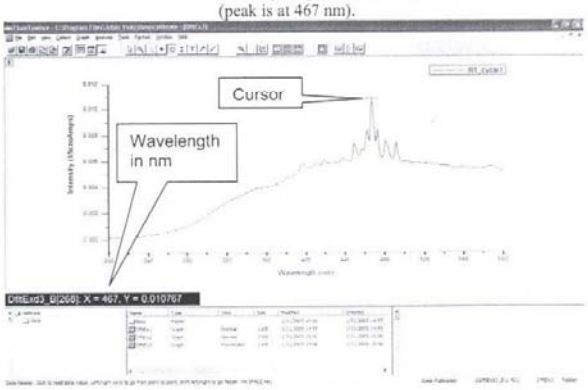


k In Peak of Interest, enter the actual or expected position of the peak (it ought to be 467 nm).

l Click OK.

m At the bottom right of the **Real Time Control** window, click Cancel.

n In the **Experiment** window, click Run to confirm the correct peak position. A correct scan is shown below (peak is at 467 nm).



Emission Calibration

Monochromator parameters for the water-Raman scan:

Monochromator (1200 grooves/mm)	Initial wave-length	Final wave-length	Incre-ment	Slits (band-pass)
Excitation	350 nm	--	--	5 nm
Emission	365 nm	450 nm	1 nm	5 nm

Detector parameters for the water-Raman scan:

Detector (Signal)	Integration time	Units
Signal (S1)	100 ms	CPS
Reference (R1)	100 ms	mA

1 Insert a cuvette with HPLC-grade, triple-distilled water in the sample compartment.

2 Close the sample compartment's lid.

3 Start FluorEssence™.

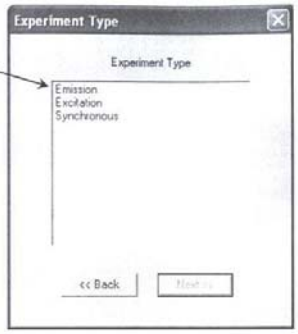
4 In the main FluorEssence window, choose the Experiment Menu button.

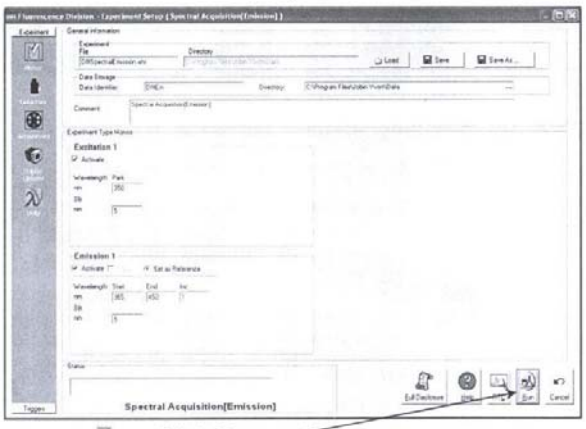
The **Fluorescence Main Experiment Menu** appears.

5 Choose Spectra.

The **Experiment Type** window appears:

6 Choose Emission.
The water-Raman experiment automatically loads.





7 Click Run.
The **Intermediate Display** opens. The water-Raman scan runs.

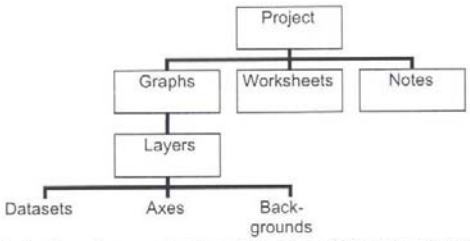
8 If the water-Raman scan is not at 397 nm, calibrate the emission monochromator as shown on pages 31–34.

Projects and files

What is a project?

A project is a collection of data that contains:

- Graphs (visual diagrams of the data)
- Worksheets (tables of data)
- Notes (comments about the data)



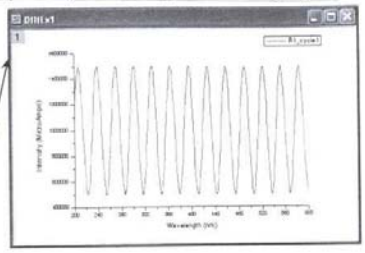
Graphs themselves may contain multiple kinds of information, including separate layers describing the data, the axes, the background colors, etc.

Concerning worksheets, a dataset must contain at least two columns, corresponding to *x*-*y* data pairs. Multiple *y* columns may correspond to a single *x* column.

Note: *For greater detail about projects, graphs, layers, and how to merge, combine, and separate them, see the Origin® on-line help files.*

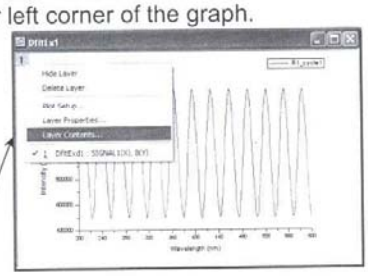
Combining two plots (datasets) into one graph

In its upper left corner, an open graph has a small box with a number in it.



1 Right-click on the numbered box in the upper left corner of the graph.

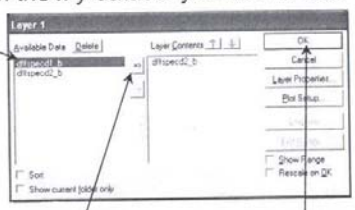
A drop-down menu appears.



2 Choose Layer Contents....

The **Layer Number** window opens. In the Available Data column, a list of *x-y* data available to plot is shown.

3 Click on the *x-y* dataset you wish to add to the plot.



Note: *For datasets with multiple y columns with one x column, the first y column plus the x column is listed as ***_a, and the second y column plus the x column is listed as ***_b, etc.*

4 Choose => to add this dataset to the plot.

The dataset appears in the Layer Contents column.

5 Adjust its order (top to bottom) with the ↑ and ↓ buttons.

6 Click OK to cause the dataset to appear in the graph.

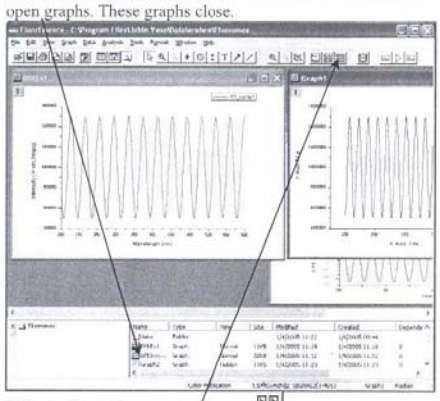
The **Layer Number** window closes.

Merging two or more graph windows

This puts all the open layers on one single page.

1 Close all graph windows you don't want to merge.

In the Project Explorer at the bottom of the main **Fluoresence** window, double-click on the names of the undesired open graphs. These graphs close.

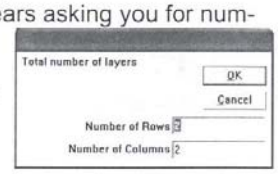


2 Click the Merge button.

All the windows are combined onto one sheet of paper. This is called a "graph". To preserve the old graphs while creating a new, overlaid version, answer Yes to the question, "Do you wish to keep the old graphs?"

3 A window appears asking you for numbers of rows and columns.

To exactly overlay the graphs, choose 1 row and 1 column.



Note: *Each layer has its own number in the upper left corner.*

Splitting two graphs by extraction

This extracts each plot to a separate layer in the graph.

1 Click on the desired plot to activate it.

2 In the toolbar, choose the Extract to Layers button.

Note: *Other buttons available using the* Customize Toolbar *command are the button for splitting each layer into a separate graph window, and the button for merging all open graph windows into one graph. See the Origin® on-line help for more information.*

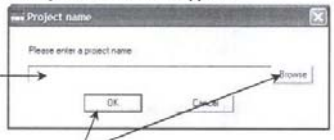
Saving and recalling a file

To save an experiment, when in a new, untitled experiment

Note: To determine if you are in an untitled, new experiment, examine the path shown at the top of the main FluorEssence™ window. It should show the word "UNTITLED" at the end of the path.

1 Run the experiment.
When the experiment is complete, the **Intermediate Display** disappears. The **Project Name** window appears.

2 Enter a new name for the project, or browse for an existing one.



Note: If you are using an existing project name, the software will allow you to overwrite existing data, or append the new data to the project.

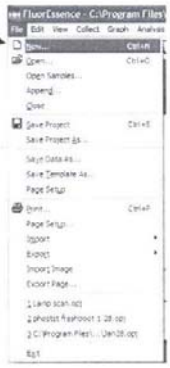
3 Click OK.
The path of the project appears at the top of the main **FluorEssence™** window. The data are now saved.

To save data into a new project when another project is already open

1 Run the experiment.

2 Choose File.
The File menu opens:

3 Choose New.



The **New** window opens:

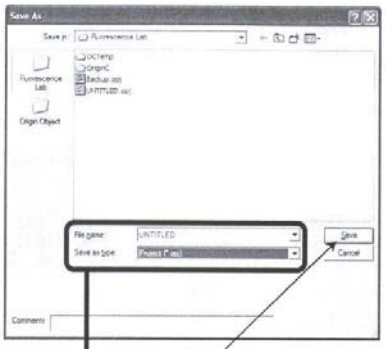
4 Choose Project from the list of objects to create.

Note: Only a Graph or a Matrix lets you pick a new Path and Name.

5 Click OK.
The data are now in an untitled project. You must next create the name for the file.

6 Choose File again.
The File menu opens.

7 Choose Save Project As....
The **Save As** window appears:



8 In the File name field, enter a name. In the Save as type field, choose Project (*.opj) from the list.

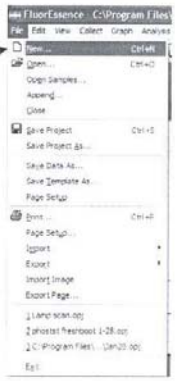
9 Click Save.
Now the new project has a name.

To recall and open an existing project

1 Click File.
The File menu opens.

2 Choose Open....
The **Open** window appears.

3 Browse for the desired project.

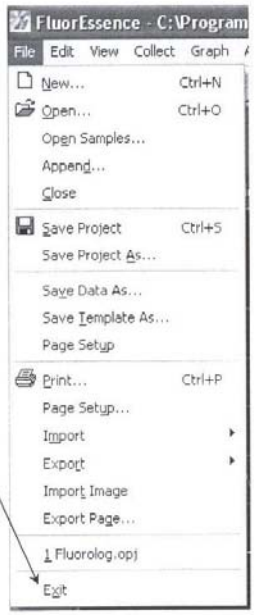


4: Shutting Down FluorEssence™

1 Save experiment files (and data files, if created).

2 In the **Experiment Setup** window, click the Close button ☒.

3 Close the **FluorEssence** window, using either the Close button ☒, or, in the File drop-down menu, Exit.

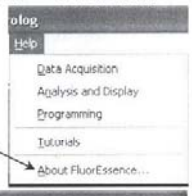


If you have a technical problem,

1. Please consult the FluorEssence™ help files and this manual, as well as all other manuals supplied with the system.

If you are unable to solve the problem,

2. Note the problem and any accompanying error messages.
3. Determine FluorEssence™'s version number.
   a. Choose the Help menu.
   b. Choose About FluorEssence....



The **About FluorEssence** window opens. Near the bottom are the FluorEssence and Origin® version numbers.

   c. Click OK to close the window.

4. Write down the software's version numbers, along with the purchase dates, model numbers, system configuration, and serial numbers of the instrument and its accessories.
5. Please contact a Spex® Fluorescence Service Department listed below.

   Be prepared to describe the malfunction and the attempts, if any, to correct it. Note any error messages observed, and have any relevant spectra (sample, polarization ratio, xenon-lamp scan, water Raman scan, etc.) ready for us to assist you.

Contact information

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E-mail	info@jobinyvon.com

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